

What is claimed is:

1. A method for translocating an RNA into a chloroplast, the method comprising:

contacting the chloroplast with an RNA comprising a first

RNA sequence and a second RNA sequence, the first RNA sequence consisting of a chloroplast localization sequence (CLS), the second RNA sequence characterized by its non-natural association with the first RNA sequence; and

translocating the RNA into the chloroplast.

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2. A method according to claim 1, wherein the CLS shares substantial homology with a viroid.

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3. A method according to claim 1, wherein the CLS consists of at least part of a viroid

4. A method according to claim 2 or 3, wherein the viroid is an *Avsunvirodiae* viroid.

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5. A method according to claim 4 wherein the viroid is an Avocado sunblotch viroid.

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6. A method according to claim 4, wherein the viroid is a peach latent mosaic virus.

7. A method according to claim 4, wherein the viroid is selected from chrysanthemum chlorotic mottle viroid and eggplant latent viroid.

8. A method according to claim 1, wherein the second RNA sequence encodes a whole or a part of a target protein.

5 9. A method according to claim 8, wherein the target protein is a herbicide-resistant protein.

10 10. A method according to claim 9, wherein the herbicide-resistant protein is selected from 5-enolpyruvylshikimate-3-phosphate synthase and acetolactate synthase.

11. A method according to claim 8, wherein the target protein is an insecticidal toxin.

15 12. A method according to claim 11, wherein the insecticidal toxin is a *Bacillus thuringiensis* toxin.

13. A method according to claim 8, wherein the protein is a marker protein.

20 14. A method according to claim 13, wherein the marker protein is green fluorescent protein.

25 15. A method according to claim 1, wherein the protein is a metabolic enzyme.

16. A method according to claim 15, wherein the metabolic enzyme is fructose 1,6-bisphosphate aldolase.

17. A method according to claim 1, wherein the second RNA sequence has a length of less than 10kb.

5 18. A method according to claim 1, wherein the RNA is a product of transcription of a DNA.

19. A method according to claim 18, wherein the DNA is located in the nucleus of a plant cell containing the chloroplast.

10 20. A method according to claim 18, wherein the DNA is located in the cytoplasm of a plant cell containing the chloroplast.

15 21. A method according to claims 19 or 20, wherein the DNA is introduced into the plant cell by a viral vector.

22. A method according to claims 19 or 20, wherein the DNA is introduced into the plant cell by a physical or chemical means.

20 23. A method according to claim 1, wherein the RNA is a product of RNA replication.

25 24. A method according to claim 23, wherein the RNA is introduced into cytoplasm of a plant cell containing the chloroplast by an RNA virus.

25. A method according to claim 1, wherein the RNA further comprises an untranslated region sequence located between the first RNA sequence and the second RNA sequence.

26. A method according to claim 1, further comprising a third RNA sequence encoding part or whole of a second protein.

5 27. A method according to claim 1, wherein the second RNA sequence in the RNA encodes a first part of a protein and wherein the chloroplast contains a second RNA, the second RNA comprising a first RNA sequence and a second RNA sequence wherein the first RNA sequence is a ribozyme sequence and the second RNA sequence encodes a second part of the protein.

10 28. A method according to claim 27, wherein the first RNA and the second RNA are *trans*-spliced to form an RNA capable of being translated into the protein.

15 29. A method according to claim 28, wherein the ribozyme is a self-splicing group I ribozyme.

20 30. A method according to claim 29, wherein the ribozyme is a *Tetrahymena thermophila* intron I *trans*-splicing ribozyme.

31. A method according to claim 27, wherein the second RNA is encoded by a DNA containing a gene fragment fused to a DNA sequence encoding the ribozyme.

25 32. A method for expressing a whole or a part of a target protein in a chloroplast, the method comprising:

contacting the chloroplast with an RNA comprising a first RNA sequence and a second RNA sequence, the first RNA

sequence consisting of a chloroplast localization sequence, the second RNA sequence encoding a whole or part of the target protein so that the first RNA chaperones the second RNA into the chloroplast; and

5 (a) expressing the whole or part of the target protein in the chloroplast.

33. An RNA comprising: a first RNA sequence which is substantially homologous to a segment of an avocado sunblotch 10 viroid (ASBVd) and is characterized by a chloroplast localizing activity and a second RNA sequence which when translated, corresponds to part or all of a protein.

34. An RNA according to claim 33, wherein the segment 15 corresponds to at least 100 nucleotides of the ASBVd.

35. An RNA comprising: a first RNA sequence which corresponds to a viroid and is characterized by a chloroplast 20 localization sequence and a second RNA sequence which when translated, corresponds to part or all of a protein.

36. A bacterial cell containing at least one RNA characterized in claim 33 or 35.

25 37. A plant cell containing at least one RNA characterized in claim 33 or 35.

38. A virus containing an RNA, or a DNA encoding the RNA of claim 33 or 35.

39. A plasmid containing a DNA sequence for transcribing the RNA of claim 33 or 35.

5 40. An RNA according to claim 33 or 35 wherein the protein is selected from a herbicide-resistant protein, a pesticide-resistant protein, a marker protein and a metabolic enzyme.

10 41. A method of expressing a protein in a plant so that undesired gene flow in the environment is prevented, comprising:

15 (a) introducing into the nucleus of the plant, a first DNA wherein the first DNA comprises a first DNA sequence and a second DNA sequence such that the first DNA sequence is transcribed to form a first RNA sequence having a chloroplast localization sequence and the second DNA sequence is transcribed to form a second RNA sequence encoding a first part of a protein;

20 (b) introducing into the chloroplast of the first plant, a second DNA, wherein the second DNA comprises a third DNA sequence and a fourth DNA sequence such that the third DNA sequence is transcribed to form a ribozyme and the fourth DNA sequence is transcribed to form a fourth RNA sequence encoding a second part of the protein;

25 (c) permitting transcription of the first DNA and its translocation into the chloroplast for *trans*-splicing of the second RNA sequence to the fourth RNA sequence for translation into the protein; and

(d) inhibiting undesired gene flow in the environment.

42. A method according to claim 41, wherein the first fusion protein of step (a) comprises a fifth DNA sequence which is transcribed to form a fifth RNA sequence which after localization in the chloroplast is spliced to a sixth RNA to form a replicase protein.

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43. A plant cell according to claim 41, further comprising a replicase translated from an exogenous nucleic acid contained in

10 the plant cell.